

DOCKET NO. HARR0018-100
Application Serial No. 09/889,761
Response to Restriction Requirement Dated December 17, 2004
Reply To Office Action Of October 19, 2004

PATENT

LISTING OF THE CLAIMS

Claim 1 (previously presented) A method of selecting a nucleic acid encoding an enzyme that is capable of converting a prodrug to its active drug form comprising the steps of:

- a) contacting a population of bacteria transformed with a bacteriophage library with a prodrug in a medium, wherein:
 - i) the transformed bacteria are in the lysogenic state, and
 - ii) when converted to its active drug form, the prodrug causes activation of the proteolytic activity of bacterial RecA and lysis of the bacteria;
- b) separating bacteriophage particles released by lysis of the bacteria from said medium; and
- c) analysing the genotype of said released bacteriophage particles for a nucleic acid encoding the enzyme.

Claim 2 (previously presented) A method of selecting a nucleic acid encoding an enzyme capable of converting a prodrug to its active drug form comprising the steps of:

- a) introducing a library of genes into bacteriophage to form a bacteriophage library;
- b) infecting a population of bacteria with said bacteriophage library;
- c) selecting said infected bacteria for bacteria in which the lysogenic state has been established;

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- d) contacting said bacteria with said prodrug in a medium;
- e) separating from said medium bacteriophage particles released by lysis of host bacteria; and
- f) analyzing the genotype of said released bacteriophage for the nucleic acid encoding the enzyme; wherein said prodrug causes activation of the proteolytic activity of bacterial RecA when converted to its active drug form.

Claim 3 (previously presented) The method of claim 1 or claim 2, wherein the steps are repeated in at least one cycle.

Claim 4 (previously presented) The method of claim 1 or 2, wherein the genotype of said released bacteriophage particles is analyzed by DNA sequencing.

Claim 5 (previously presented) The method of claim 1 or 2, wherein said bacteriophage carry a gene encoding antibiotic resistance or other selectable marker.

Claim 6 (previously presented) The method of claim 1 or 2, wherein said enzyme is selected from the group consisting of nitroreductase, flavin reductase, DT-diaphorase, thymidine kinase, cytosine deaminase, and purine nucleoside phosphorylase.

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PATENT

Claim 7 (previously presented) The method of claim 1 or 2, wherein said prodrug is selected from the group consisting of CB1954, SN 23862, 2-[N,N-bis(2-iodoethyl)amino]-3,5-dinitrobenzamide, 5-fluorocytosine, acyclovir, ganciclovir, and 6-methyl-9-(2-deoxy- β -D-erythro-pentofuranosyl) purine.

Claim 8 (previously presented) The method of claim 1 or 2, wherein said bacteriophage is the bacteriophage lambda or a lambda derivative.

Claim 9 (previously presented) The method of claim 2, wherein said gene library comprises genes encoding variants of a single enzyme.

Claim 10 (previously presented) The method of claim 9, wherein said variants comprise amino acid deletions and/or insertions and/or substitutions from the wild type enzyme.

Claim 11 (previously presented) The method of claim 9, wherein said genes encoding said variants are generated by DNA shuffling, random mutagenesis, or PCR shuffling.

Claim 12 (previously presented) The method of claim 1 or 2, wherein said activity of said bacterial RecA protein is caused by the generation of single-stranded DNA in the bacterium.

DOCKET NO. HARR0018-100
Application Serial No. 09/889,761
Response to Restriction Requirement Dated December 17, 2004
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PATENT

Claim 13 (previously presented) The method of claim 12, wherein said single-stranded DNA is generated as a consequence of the enzymatic conversion of the prodrug to its active drug form.

Claim 14 (previously presented) The method of claim 12, wherein said single-stranded DNA is generated as a result of a break in one or both strands of the DNA, a cytotoxic lesion, a DNA crosslink or a monovalent DNA adduct, or by inhibition of the progress of DNA replication.

Claim 15 (previously presented) The method of claim 1 or 2, wherein said enzyme comprises nitroreductase and said prodrug comprises CN1954.

Claim 16 (previously presented) The method of claim 1 or 2, wherein said bacteriophage is λ JG3J1.

Claim 17 (previously presented) The method of claim 1 or 2, wherein said bacteria are *E. coli* strain C600 *Hfl*.

DOCKET NO. HARR0018-100
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PATENT

Claim 18 (withdrawn) A method of cloning a nucleic acid encoding a catalytic enzyme or enzyme fragment, said catalytic enzyme or enzyme fragment being capable of converting a prodrug to its active drug form, comprising the steps of:

- a) contacting a population of bacteria transformed with a bacteriophage library with a prodrug in a medium, wherein
 - i) the transformed bacteria are in the lysogenic state, and
 - ii) when converted to its active drug form, the prodrug causes activation of the proteolytic activity of bacterial RecA and lysis of the bacteria;
- b) separating bacteriophage particles released by lysis of the bacteria from said medium;
- c) analyzing the genotype of said released bacteriophage particles for a nucleic acid encoding the enzyme, or functional fragment thereof; and
- d) cloning the nucleic acid of the released bacteriophage particles that encode the enzyme or enzyme fragment.

Claim 19 (withdrawn) A method of cloning a nucleic acid encoding a catalytic enzyme or enzyme fragment, said catalytic enzyme or enzyme fragment being capable of converting a prodrug to its active drug form, comprising the steps of:

- a) introducing a library of genes into bacteriophage to form a bacteriophage library;

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- b) infecting a population of bacteria with said bacteriophage library;
- c) selecting said infected bacteria for bacteria in which the lysogenic state has been established;
- d) contacting said bacteria with said prodrug in a medium;
- e) separating from said medium bacteriophage particles released by lysis of host bacteria;
- f) analyzing the genotype of said released bacteriophage for the nucleic acid encoding the enzyme or functional fragment thereof; and
- g) cloning the nucleic acid of the released bacteriophage particles that encode the enzyme or enzyme fragment.

Claim 20 (withdrawn) A nucleic acid molecule encoding a catalytic enzyme or enzyme fragment isolated according to the method of claim 18 or 19.

Claim 21 (withdrawn) A catalytic enzyme or enzyme fragment encoded by the nucleic acid molecule of claim 20.